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# Antibiotic sensitivity patterns of penicillinase-positive and penicillinase-negative strains of Neisseria gonorrhoeae isolated in Fukuoka, Japan

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SUMMARY Penicillinase production and minimum inhibitory concentrations (MICs) of penicillin G, amoxycillin, doxycycline, and spectinomycin were studied for 155 gonococcal strains isolated in Fukuoka, Japan, between April and December 1981. Of 155 isolates, 25 (16·1%) strains were identified as penicillinase-producing *Neisseria gonorrhoeae* (PPNG) by a rapid iodometric test. The antibiotic sensitivity of 130 penicillinase-negative (non-PPNG) and 25 PPNG strains was compared. The mean MIC of penicillin G for non-PPNG strains was 0·65 μg/ml. Twenty-five PPNG strains had MICs of penicillin G between 6·25 and >100 μg/ml. The susceptibility to amoxycillin and doxycycline of PPNG strains was significantly lower than that of non-PPNG strains. All the strains proved to be sensitive to spectinomycin. The MICs of four antibiotics for non-PPNG strains were significantly correlated.

## Introduction

Over the past 20 years there has been a progressive increase in the prevalence of strains of *Neisseria gonorrhoeae* showing resistance to penicillin.[1-5] *N gonorrhoeae* was previously susceptible to penicillin. Both the frequency of occurrence and the degree of resistance of strains of the organism, however, have increased very slowly, as is reflected in the minimum inhibitory concentrations (MICs) of penicillin.[1-5]

Penicillinase-producing *N gonorrhoeae* (PPNG) strains were isolated for the first time in 1976 in the United Kingdom[6-8] and in the United States[9] and since then have been found in many countries.[10-21] An especially high incidence of PPNG has been reported from countries in South-east Asia, such as Malaysia, Thailand, and the Philippines.

We report the trends in antibiotic susceptibility of gonococci and the incidence of PPNG in Fukuoka City, Japan.

## Materials and methods

Specimens were collected from patients with gonorrhoea who attended Urabe Hospital between April and December 1981. Specimens were taken from the urethra in men and from the urethra, vagina, and endocervix in women. Gram-stained smears of exudate were examined microscopically and *N gonorrhoeae* was cultured on GC medium base (Difco) enriched with 1% bacto-haemoglobin and 1% Isovitalex (BBL) supplemented with 0·2 μg/ml lincomycin and 2 units/ml colistin.

### IDENTIFICATION OF GONOCOCCI

After incubation for 24 hours at 37°C in candle-extinction jars colonies of organisms were examined by Gram stain microscopy and oxidase reaction; their ability to produce acid from glucose, lactose, maltose, and sucrose was tested in the Minitek system (BBL). Their ability to reduce nitrate was also evaluated.

### ANTIBIOTIC SUSCEPTIBILITY TEST

In-vitro antibiotic testing of the gonococcal isolates was performed by the plate dilution method. Using a spectrophotometer bacterial suspensions were adjusted to $1 \times 10^6$ organisms/ml and one loopful (about $1 \times 10^4$ colony-forming units) was streaked on to GC agar plates containing serial dilutions of antibiotics in concentrations of 0·025-100 μg/ml. The plates were incubated for 24 hours at 37°C in a candle jar. The concentration of antibiotic required to produce total inhibition of growth constituted the

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minimum inhibitory concentration (MIC) for the isolates tested.

RAPID IODOMETRIC METHOD

The production of β-lactamase by gonococcal isolates was examined by a rapid iodometric method.[22]

STATISTICAL ANALYSIS

The difference between the mean MICs of antibiotics was tested by the Mann-Whitney U-test. Rank correlation coefficient between paired antibiotics was measured by Spearman's method and the significance of correlation coefficient evaluated. MICs of <0·025 and >100 μg/ml were included in the values for 0·025 and 100 μg/ml respectively.

## Results

Of 155 isolates of *N gonorrhoeae*, 25 (16·1%) produced penicillinase.

The susceptibility of isolates to penicillin G, amoxycillin, doxycycline, and spectinomycin was measured in concentrations ranging from 0·025 to 100 μg/ml. The MICs of these antibiotics for 130 penicillinase-negative (non-PPNG) and 25 PPNG strains are shown in table I. The mean MIC for penicillin G of non-PPNG strains was 0·65 μg/ml. Only 22 of 155 (14·2%) isolates were inhibited by ≤0·1 μg/ml of penicillin G. Sixty-nine of 155 (44·5%) strains required ≥1·56 μg/ml of penicillin G to be inhibited. One non-PPNG strain had an MIC of penicillin G of 50 μg/ml; its MICs for amoxycillin, doxycycline, and spectinomycin were also high (6·25, 50, and 25 μg/ml respectively). PPNG strains had MICs of penicillin G between 6·25 and >100 μg/ml and of amoxycillin between 12·5 and >100 μg/ml.

There was a significant difference between the mean MIC of doxycycline for PPNG and that for non-PPNG strains. No significant difference was found, however, between the mean MIC of spectinomycin for PPNG and that for non-PPNG strains. Spectinomycin-resistant strains were not detected. Rank correlation coefficients for paired antibiotics were calculated using MICs for non-PPNG strains (table II). Moderate correlations were found for susceptibility to penicillin G, amoxycillin, and doxycycline, while lower correlations usually occurred with the antibiotics paired with spectinomycin.

TABLE II *Rank correlation coeffficients (r) of the in-vitro susceptibility of N gonorrhoeae to antibiotics*

| *Antibiotics* | *r* | *Probability* |
|---|---|---|
| Penicillin G and amoxycillin | 0·687 | P<0·001 |
| Penicillin G and doxycycline | 0·611 | P<0·001 |
| Penicillin G and spectinomycin | 0·246 | 0·001<P<0·01 |
| Amoxycillin and doxycycline | 0·379 | P<0·001 |
| Amoxycillin and spectinomycin | 0·396 | P<0·001 |
| Doxycycline and spectinomycin | 0·209 | 0·01<P<0·05 |

## Discussion

In this study we found that the occurrence of less sensitive and resistant strains of *N gonorrhoeae* to penicillin was very high in Fukuoka City. Of 155 isolates, 69 (44·5%) required ≥1·56 μg/ml of penicillin G to be inhibited. Moreover, 25 of 155 (16·1%) isolates were identified as PPNG. The mean

TABLE I *In-vitro susceptibility of isolates of N gonorrhoeae to antibiotics*

| | *Penicillin G* | | *Amoxycillin* | | *Doxycycline* | | *Spectinomycin* | |
|---|---|---|---|---|---|---|---|---|
| *MIC (μg/ml)* | *Non-PPNG* | *PPNG* | *Non-PPNG* | *PPNG* | *Non-PPNG* | *PPNG* | *Non-PPNG* | *PPNG* |
| <0·025 | 1 | | 1 | | 1 | | | |
| 0·025 | 1 | | | | | | | |
| 0·05 | 6 | | 2 | | | | | |
| 0·1 | 14 | | 3 | | | | | |
| 0·2 | 16 | | 4 | | | | | |
| 0·39 | 28 | | 20 | | 10 | | | |
| 0·78 | 20 | | 36 | | 11 | | | |
| 1·56 | 10 | | 45 | | 30 | 3 | 6 | 1 |
| 3.13 | 18 | | 18 | | 22 | 1 | 8 | 3 |
| 6·25 | 12 | 4 | 1 | | 15 | 2 | 8 | |
| 12·5 | 3 | 2 | | 2 | 18 | 7 | 53 | 5 |
| 25 | | 5 | | 3 | 8 | 5 | 54 | 16 |
| 50 | 1 | 6 | | 3 | 12 | 7 | 1 | |
| 100 | | 7 | | 5 | 3 | | | |
| >100 | | 1 | | 12 | | | | |
| Total No of strains | 130 | 25 | 130 | 25 | 130 | 25 | 130 | 25 |
| Mean MIC (μg/ml) | 0·65 | 36·20 | 0·98 | 67·56 | 4·22 | 14·70 | 13·72 | 15·76 |
| U-test* | P<0·001 | | P<0·001 | | P<0·001 | | NS | |

NS = not significant; PPNG = penicillinase-producing *N gonorrhoeae*
*Mann-Whitney U-test

MIC of penicillin G was 0·65 μg/ml for all except penicillinase-producing strains. The first infection due to PPNG in Japan was reported by Onoda *et al*[18] in 1979; the actual date of isolation was 1977. In this study almost all infections with PPNG strains were acquired around Fukuoka City. The incidence of PPNG strains in Fukuoka City was lower than that of Thailand (1977-79; 18·5%)[14] and Singapore (1979; 19·2%)[20] but higher than that of Riyadh (1979-80; 12%).[13] The epidemiology of gonorrhoea caused by PPNG strains will be reported in detail elsewhere.

Recently, Shtibel[23] reported two strains of gonococci showing high resistance to penicillin; the MIC of penicillin for both strains was 50 IU/ml. In our present study one of the non-PPNG strains showed high resistance to penicillin G (MIC of penicillin 50 μg/ml). The occurrence of strains of *N gonorrhoeae* which are highly resistant to penicillin but which did not produce β-lactamase has to be considered when chemotherapy is being planned.

There was a significant difference between the mean MIC of doxycycline for PPNG and that for non-PPNG strains. The reason for this is not clear at present. A significant difference was not observed, however, between the mean MIC of spectinomycin for PPNG and that for non-PPNG strains.

A relatively high correlation between the sensitivity to penicillin G and doxycycline was observed among non-PPNG strains. These two antibiotics are structurally different but both have small molecular weights and are hydrophilic. They are thought to pass through the porin proteins in the outer membrane.[24] Alteration of the porin proteins of gonococci may modify the susceptibility to these two antibiotics.

Recently, spectinomycin-resistant gonococcal strains have been reported.[25] Such strains were not, however, observed in this study.

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